Claims

1. An assay method for the determination of transcobalamin II (TCII) bound cobalamin in a body sample, comprising contacting a cell free sample of a body fluid with an immobilised or immobilizable specific binding ligand for TC II or cobalamin bound TCII (holo TC II), separating a ligand bound fraction from a non-ligand bound fraction and measuring the holo-TC II or TC-II bound cobalamin content therein.

2. An assay method as claimed in claim 1 wherein said specific binding ligands for TC II or holo-TCII allow for separation and concentration of the TC II or holo-TC II in the sample of at least 3-fold and up to greater than 10-fold.

3. An assay method as claimed in claim 1 or claim 2 wherein said assay is capable of detecting holo-TC II at a concentration as low as 9 pM.

4. An assay method as claimed in ~~any one of~~ claims 1 ~~to 3~~ wherein said specific binding ligand is selected from the group comprising a polyclonal or monoclonal antibody, an antibody fragment, a polypeptide, an oligopeptide, a small organic chemical, a specific binder selected from a combinatorial chemistry or phage display library, a specifically binding sequence of DNA or RNA, or a cell surface receptor.

5. An assay method as claimed in ~~any one of~~ claims 1 ~~to 4~~ wherein said specific binding ligand exhibits a high degree of selectivity and specificity towards TC II and exhibits low affinity towards other TC proteins, in either apo or holo form, or any other cobalamin-binding protein.

6. An assay method as claimed in ~~any one of~~ claims 1 ~~to 5~~ wherein said cobalamin is released from the holo TCII molecules by changing the temperature or the pH of the surrounding medium.

7. An assay method as claimed in claim 6 wherein said released cobalamin is determined by a competition assay performed by contacting an immobilised binding partner for cobalamin with the dissociated cobalamin of the sample in the presence of labelled ligand which competes with the isolated cobalamin for binding to the immobilised binding partners.

8. An assay method as claimed in ~~any one of~~ claims 1 ~~to 7~~ wherein said method comprises contacting a solid support having immobilised thereon a TC II or holo-TC II binding ligand, with the sample under investigation and also with a non-immobilised ligand,

wherein said immobilised ligand is capable of binding to TC II or holo-TC II, to said non-immobilised ligand or to complexes of said TC II or holo-TC II and said non-immobilised ligand, and said non-immobilised ligand is capable of binding to at least one of said immobilised ligand, TC II or holo-TC II and complexes of said immobilised ligand and TC II or holo-TC II;

wherein if said assay method is a sandwich assay, at least one of said ligands is specific for holo-TC II and if said assay is a competition assay said immobilised ligand is specific for holo-TC II and competitors thereof;

whereby the proportion of said immobilised ligand bound by TC II or holo-TC II, by said non-immobilised ligand or by complexes of said non-immobilised ligand and TC II or holo-TC II is dependent on the amount of holo-TC II present in said sample, and,

said non-immobilised ligand is capable of generating a directly or indirectly detectable signal

when bound or when unbound;

separating a bound fraction from a non-bound fraction; and

directly or indirectly determining the non-immobilised ligand bound to the immobilised ligand (the bound fraction) or non-bound and in solution (the non-bound fraction);

where the contacting of the sample and said non-immobilised ligand with the solid support may be performed separately, simultaneously or sequentially, and if performed separately or sequentially, they may be contacted in either order.

9. An assay method as claimed in ~~any one of~~ claims 1 ~~to 8~~ wherein a preliminary separation step is carried out using cobalamin or an analogue or fragment thereof which selectively binds the apo-forms of both TC II and haptocorrin (HC), such that the apo forms of the TC II and HC proteins are bound by the cobalamin, analogue or fragment thereof and separated from the holo-TC II and holo-HC complexes.

10. An assay method as claimed in claim 9 wherein the binding of apo TC II and apo HC to cobalamin, analogues or fragments thereof takes place at a site or in such a manner which inhibits subsequent recognition and binding of the immobilised cobalamin bound TC II by the non-immobilised ligand or binding partner for TC II.

11. An assay method as claimed in ~~any one of~~ claims 1 ~~to 10~~ wherein said non-bound fraction is at least 80%, 90% or 95% free of either TC II or holo-TC II.

12. An assay method as claimed in ~~any one of~~ claims 1 ~~to 11~~ further comprising a preliminary separation step in which the sample is contacted with an immobilized or immobilizable specific binding ligand for haptocorrin.

13. An assay method as claimed in ~~any one of~~ claims 1 ~~to 12~~ wherein said TC II binding ligand possesses an affinity constant of at least 10^9M^{-1}.

14. An assay method as claimed in ~~any one of~~ claims 1 ~~to 13~~ wherein the affinity constant is greater than $10^{11}M^{-1}$.

15. An assay method as claimed in ~~any one of~~ claims 1 ~~to 14~~ wherein the degree of cross-reactivity of a holo-TC II or TC II binding ligand with HC is between 0.1% and 1%.

16. An assay method as claimed in ~~any one of~~ claims 1 ~~to 15~~ wherein the degree of cross-reactivity of a holo-TC II or TC II binding ligand with HC is less than 0.1%.

17. An assay method as claimed in ~~any one of~~ claims 1 ~~to 16~~ wherein said sample comprising the holo-TC II complex is contacted with a solid phase to which a labelled ligand recognising the same binding sites on the immobilised ligands as holo-TC II is bound; holo-TC II in said sample competes with said bound labelled ligand for said binding sites such that after equilibration of the system, there is a directly proportional relationship between the amount of labelled ligand displaced from said solid support and detectable in solution and the amount of holo-TC II present in the original sample; said labelled ligand being detected directly or indirectly as the amount of labelled ligand bound or not bound to said solid support as appropriate.

18. An assay method as claimed in ~~any one of~~ claims 1 ~~to 17~~ wherein said holo-TC II containing sample is contacted with a solid support having holo-TC II immobilised thereon and a labelled non-immobilised holo-TC II specific binder, wherein free holo-TC II in the

sample and immobilised holo-TC II compete for binding with the labelled non-immobilised ligand; and determination of the labelled ligand bound to the solid phase or remaining in solution allows determination of the holo-TC II concentration.

19. An assay method as claimed in ~~any one of~~ claims 1 ~~to 18~~ wherein said holo=TC II containing sample is contacted with labelled holo-TC II and immobilised ligand therefor; said labelled and non-labelled holo-TC II complexes compete for binding to the immobilised ligand and after equilibrium is reached, the amount of labelled holo-TC II bound to the immobilised ligand is indirectly proportional to the amount of holo-TC II in the sample.

20. An assay method as claimed in ~~any one of~~ claims 1 ~~to 19~~ wherein said body sample is selected from the group comprising seminal fluid, cerebro-spinal fluid, amniotic fluid or a blood derived sample.

21. An assay method as claimed in claim 20 wherein said blood derived sample is serum or plasma.

22. An assay method as claimed in ~~any one of~~ claims 1 ~~to 21~~ wherein said bound fraction is separated from said unbound fraction by precipitation, centrifugation, filtration or chromatographic methods.

23. An assay method as claimed in ~~any one of~~ claims 1 ~~22~~ wherein said detectable ligand is labelled with a signal forming label which may be determined by luminescence, chemiluminescence, colorimetric assessment, fluorescence, radioactivity or by enzymic activity.

24. An assay method as claimed in ~~any one of~~ claims 1 ~~to 23~~ in which assay calibration is effected using a holo-TC II standard.

25. An assay as claimed in claim 24 wherein said standard is human, native or recombinant holo-TC II.

26. A kit for use in a diagnostic assay according to ~~any one of~~ claims 1 ~~to 25,~~ comprising:

an immobilized or immobilizable specific binding ligand for TC II or holo-TC II;

preferably a holo-TC II solution of known concentration or a set of such solutions having a range of holo-TC II complex concentrations;

optionally, a release agent to release cobalamin from holo-TC II; and

optionally a labelled ligand.

27. The use of holo-TC II as a calibrator in an assay for holo-TC II.